Welcome to BIOC 318, Laboratory Studies in Applied Microbiology

Many studies of samples taken from a wide variety of sources have revealed that between 0.01 and 0.1% of all bacterial cells that can be seen in a light microscope are "uncultivable" in the laboratory.[1] This means that for every bacterial species successfully isolated from an environmental sample, as many as 10,000 species remain undetected. Scientists have estimated that up to one trillion microbial species exist on Earth, about 0.001% of which are actually known.[2] If the one trillion estimate is accurate, then if 100 Rice students *each* discover 100 new species every year (obviously not a realistic expectation), it will take 100 million years to discover all of them.

Why should these findings interest us? It means that the opportunities for discovery are wide open. If you can overcome the significant challenges that accompany the search for new bacterial species you may indeed go where no one else has gone.

Our projects

Example of some of the work you will conduct in this course. (A) Agar plates with bacterial unknowns, 72h following aerobic incubation at 30˚C. *(B)* Identically streaked agar plates incubated anaerobically for 72h, revealing relationship to oxygen.

After an afternoon introduction and initial training, we will jump right into it. As a class we will isolate as many unique species as we can from a variety of soil samples, collected locally. In teams you will conduct initial observations and assays in order to narrow the possible taxa to which each isolate belongs. From there you will need to choose a project.

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[1] D'Onofrio, Anthony, Jason M. Crawford, Eric J. Stewart, Kathrin Witt, Ekaterina Gavrish, Slava Epstein, Jon Clardy, and Kim Lewis . "Siderophores from Neighboring Organisms Promote the Growth of Uncultured Bacteria." Chemistry & Biology (2010): 254-64.

[2] Offord, Catherine. "Earth: Home to 1 Trillion Microbial Species." The Scientist (2016): http://www.the-scientist.com/?articles.view/articleNo/46016/title/Earth--Home-to-1-Trillion-Microbial-Species/

Project "X" – Isolate and identify species of *Streptomyces* bacteria, to support studies of interactions between different strains. It has been proposed that positive and negative interactions among strains define much of the ecology of soil. The ultimate goal is to discover how some of these interactions may have evolved. This project will have you working with people in the Shamoo lab.

Project "Y" – Attempt to isolate currently "uncultivable" species by (1) applying recently developed published methods and/or (2) developing and applying methods of our own. Most of the work on this project will have you attempt to obtain cultures that may represent rare species. You will prepare samples for 16S rRNA sequencing followed by a BLAST search for a match to known species. If there are no close matches, then you may have succeeded!

Project "Z" – The accuracy of the 16S rRNA sequencing method for bacterial identification is only as good as its database. For this project you will select an isolate, obtained by conventional culture methods, and employ two approaches to identify it to the species level. You will make observations and conduct assays to characterize the isolate by phenotype and conduct 16S rRNA sequencing and a BLAST search for comparison.

All of these projects will require a writeup at the end of the course.

Objectives and learning outcomes

The main goal of this course, as must be true for any experiential course, is personal development. We want you to build on skills that you've already acquired and learn new ones. Below we summarize our most important learning objectives and outcomes.

1. Learn to work as a productive member of a research team on a long term project
2. Be able to access and employ published reference materials, current primary literature, and other information sources in order to advance your understanding of the properties of bacteria and how to isolate and characterize them
3. Learn to integrate your previous and newly acquired experience into development of new techniques and strategies
4. Improve your powers of observation, such as the ability to use physical and metabolic differences to distinguish strains and species of bacteria and to draw information from microscopic observations
5. Learn to make the decisions that are necessary in order to conduct a systematic open-ended investigation
6. Learn to make your work accessible to yourself and others by documenting your activities and findings in a well organized laboratory notebook, by contributing to our laboratory's database, and by summarizing your work in a clearly written report

7. Learn and practice good laboratory "citizenship," including respect for people, the facility, and equipment
8. Acquire proficiency in essential methodologies and use your newly acquired skills to advance our project
9. Learn to appreciate the value and potential of pure science, that is, the pursuit of knowledge for its own sake

Please see the Syllabus for course details, course timetable, and a list of assignments with due dates.

Welcome again to BIOC 318. On the home page you will find a brief introduction to the course and a description of the overall course objectives and expected learning outcomes. In addition, please look over the following information about the course, instructors, and policies. Following the course policies is a complete timetable of scheduled activities and assignments with dates, times, and locations when applicable.

*****The information contained in this syllabus and related pages may be subject to change, with reasonable notice, at the discretion of the instructor.*****

Instructor and office hours

David Caprette, Teaching Professor
ABL 327 – third floor Anderson Biological laboratory Building
caprette@rice.edu, 713-348-3498 (campus extension 3498)
Office hours are variable (see HERE for a current schedule)

Preparing for the course

Please plan to apply the skills and best practices that you learned in Bioc 211, Intermediate Experimental Biosciences (course prerequisite) and in Bioc 311, Advanced Experimental Biosciences, if you have had the opportunity to complete that course. Please check the Course Schedule (below), review the meeting schedule for your lab section, and block out the scheduled times on your calendar. You must not schedule events that conflict with any of the listed in-lab activities, nor let anyone schedule a conflicting event for you.

Required materials

You will need eye protection for this course, consisting of a pair of lab goggles or laboratory safety glasses with side protection. A lab coat will be provided. There is no textbook but you will need to obtain a laboratory notebook. You are welcome to start with a partly filled notebook from another course, even one obtained from another student. New notebooks can be purchased from the campus store or ordered directly from the distributor. Please purchase a Hayden-McNeil Carbonless

TOP Permanent Lab Notebook, 50 Set or 100 Set from Amazon, from WH Freeman, or the Campus store (ISBNs below).

Chemistry Top Bound 100 pages, ISBN 978-1-930882-00-3
or
Chemistry Top Bound 50 pages, ISBN 978-1-930882-50-8

Course timetable

All meetings begin at 1 pm unless otherwise noted. All meetings will be held in ABL 127. Documents to help you prepare for lab (listed under the heading "Preparation" below) can be found in Files and are also accessible from the side menu on the BIOC 318 web pages.

To print a file, download it first then print as usual. Attempting to print directly from a Canvas window may not work.

Sec 001	Sec 002	Preparation	Description of activities
Weds Oct 10, 1:00 pm	Thur Oct 11, 1:00 pm	• Review the course home page in Canvas and this syllabus and have questions ready • Review the documents "Aseptic technique," "Making serial dilutions," and Preparing spread plates," and be prepared for a quiz on the material; all documents are in Files	• Introductory classroom meeting • Short quiz, lab tour, and practice sessions for dilutions and spreading plates
Mon Oct 15	Tues Oct 16	• Review the documents "Isolating colonies" and "Colony morphology;" be prepared for a brief quiz • Review the PowerPoint slides in "Course introduction," posted in Files • Peruse the document "Culture media;" you will not be quizzed on this material	• Prepare agar media and pour plates to be used for isolating colonies • Dilute and spread water samples • Practice sessions on isolating colonies and making streak plates
Weds Oct 17	Thur Oct 18	• Review the document "Agar slant tubes"	• Prepare agar media for slant tubes • Identify and describe unique colonies on

			spread plates, streak out onto fresh agar plates
Mon Oct 22	Tues Oct 23	• Review the documents "Gram stain," and "Relationship to oxygen," and be prepared for a short quiz	• Select a project, organize project teams, begin planning • Consolidate isolates, conduct anaerobic incubations • Practice making Gram stains • (Tues section only) Gram stain isolates on aerobically incubated plates from the Monday group
Weds Oct 24	Thurs Oct 25	• Again review the document "Relationship to oxygen" • Peruse the document "Using inoculating sticks;" you may prefer this technique over the technique that employs a flame and inoculating loop • Review the document "Using Bergey's manuals"	• Sign out a Bergey's manual • Evaluate anaerobically incubated plates for growth • (Weds section only) Gram stain isolates on aerobically incubated plates from the Tuesday group • (Thursday section only) Learn and practice microscopic examination of Gram stains • Assemble notebook copy pages, turn in for mid-course evaluation
Mon Oct 29	Tues Oct 30	• As you work with the microscope you may want to consult the document "Viewing Gram stains," which includes a step by step guide to using our Leica compound microscopes	• Continue project work • (Wednesday section only) Learn and practice microscopic examination of Gram stains • Learn and practice microscopic observation of living bacteria • In teams, begin to assign isolates to Bergey's "Groups" • We may want to conduct catalase and oxidase tests to help assign

			groups
Weds Oct 31	Thurs Nov 1	• Enter observations for your isolates into the spreadsheet in Google Sheets	• Continue to record observations from microscopic examination of wet mounts from fresh cultures of your isolates • Conduct oxidase and catalase tests on all isolates (if not done already)
Mon Nov 6 thru Weds Nov 29	Tues Nov 7 thru Thurs Nov 30	• Continue to assign isolates to Bergey's Groups, using recorded observations and in consultation with team members • Guided by Bergey's tables, plan strategies for identifying isolates • Peruse the following resources as needed: "Assays," "Assays illustrated," "Characterization writeup," and "Project proposal guidelines"	• Conduct project work • Complete and submit the writeup

Course grade

Your grade will be based upon your preparation and lab performance (20%), self reliance and contribution to team efforts (10%), laboratory notebook (30%), and your final write-up (50%).

Additional information and policies

Absences. Because the work in this course is part of a long term goal-oriented project, the concept of a "make-up lab" does not apply. Missing an important event is a lost opportunity that cannot be made up. As far as your course grade is concerned, absences from required meetings will be excused for the usual reasons, e.g., illness or family emergency. Absences due to circumstances within a student's control (e.g., forgetting to show up or travel for interviews) cannot be excused and will be reflected in the performance grade.

Lab safety. All participants must engage in safe laboratory practices and abide by specific "rules" set down for this course.

Late assignments. An assignment that is submitted late will lose 10% per day including holidays and weekends.

Honor code policy. As with all biosciences courses, the Rice University Honor Code applies to all personal conduct and assignments. All work submitted under your name must be your own work, not copied from another source nor completed with unauthorized assistance. Compliance with the honor code includes providing accurate information when requested, such as honest evaluations of yourself and your teammates.

Students with disabilities. Any student with a disability needing academic adjustments or accommodations should contact Dr. Caprette as soon as possible and also contact Disabled Student Services in the Ley Student Center.